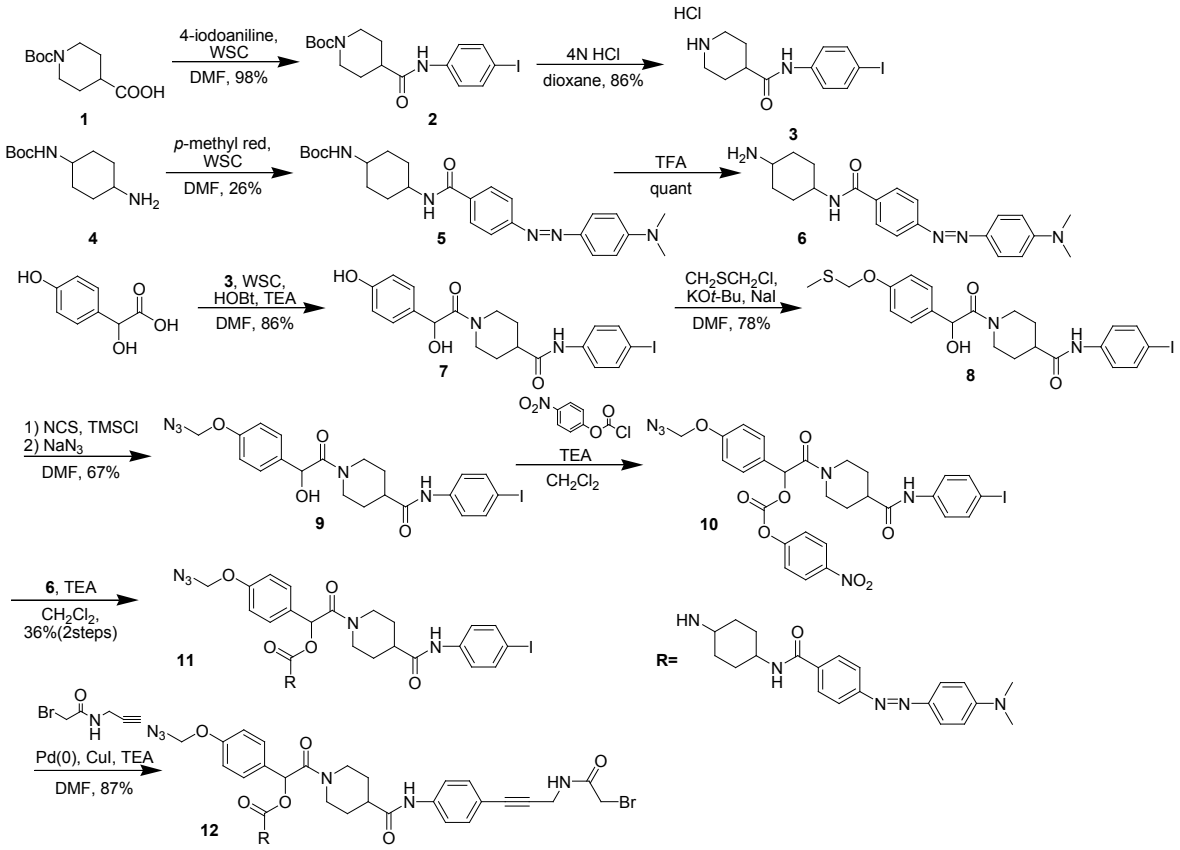
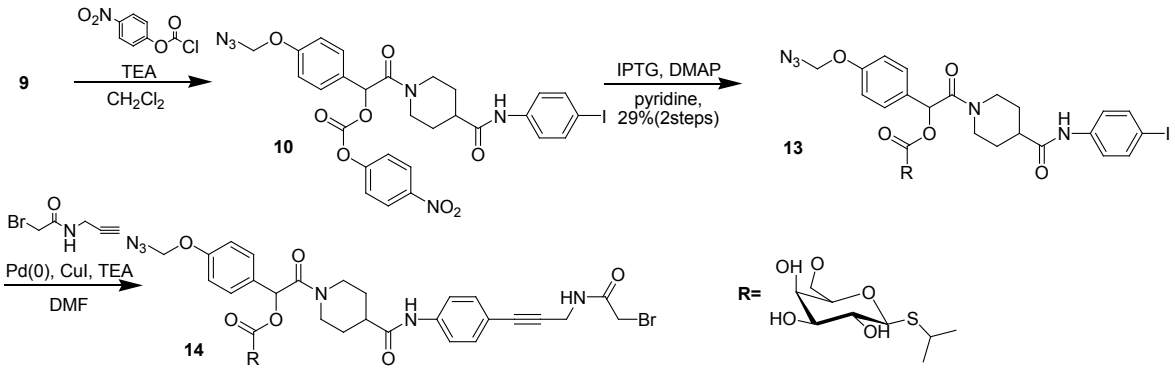
Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Contents



Scheme S1. Synthesis of the azidomethyl-caged immolative linker **12**.



Scheme S2. Synthesis of the azidomethyl-caged immolative linker **14**.

ON **1**: 5’-d(TGAAG^{5Flu}**T**GCTT)-**MR**-3’

ON **2**: 5’-**TPP**-d(TGTGGGCA)-3’

ON **3**: 5’-**TPP**-d(TGTGGGCAA)-3’

ON **4**: 5’-**TPP**-d(TGTGGGCAAG)-3’

ON **5**-**9**: 5’-d(ACTCTTGCCCACA(T)$_n$AAGCACTTCAGCG)-3’

ON **5**: n=0, **6**: n= 1, **7**: n= 2, **8**: n= 3, **9**: n= 4

ON **10**: 5’-d(ACTCTTGCCCACATAAGAACTTCAGCG)-3’

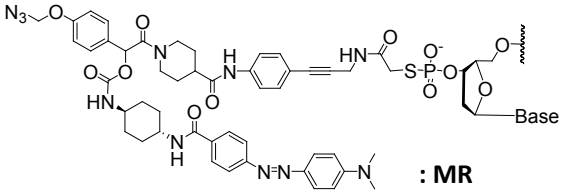
ON **11**: 5’-r(ACUCUUGCCCACAUAAGCACUUCAGCG)-3’

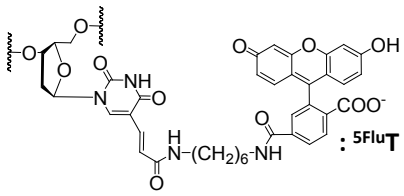
ON **12**: 5’-d(GCTGGCGGTC^{5Flu}**T**GGGT)-**MR**-3’

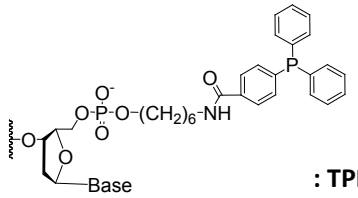
ON **13**: 5’-d(GCTGGCGGTCTGGGT)-**IPTG**-3’

ON **14**: 5’-**TPP**-d(GTTTCCCTCTTCACG)-3’ (match probe)

ON **15**: 5’-**TPP**-d(GCCTTCTCCCGAAGT)-3’(scramble probe)







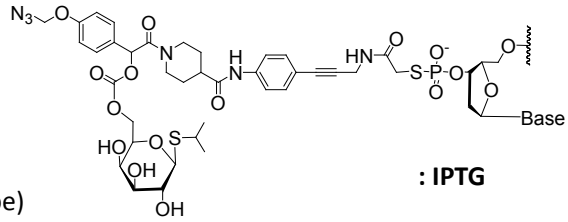


Figure S1. Sequences of oligonucleotides used in this study. ON **1** and **12** were modified with Fluorescein-dT (^{5Flu}T) and compound **12**(MR). ON **2**-**4**, **14** and **15** were modified with TPP at the 5’ terminal. ON **13** was modified with compound **14** (IPTG) at the 3’ terminal. The target sequence is underlined. The mismatch base is red.

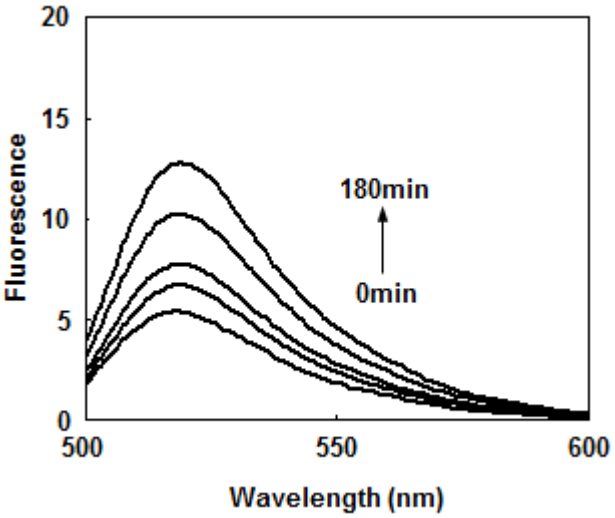


Figure S2. Fluorescent emission spectra with DTT. The reaction were carried out the following conditions. 20 mM Tris-HCl buffer(pH7.2) containing 50 nM ON **1** with 100 mM DTT. Excitation wavelength: 490 nm.

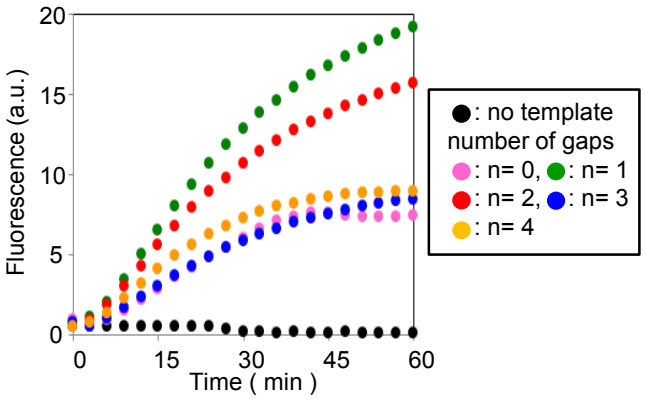


Figure S3. Time course of the fluorescence intensity in the reaction between 50 nM ON **1** and 250 nM ON **4** with 0 or 50 nM target DNA (ON **5**-**9**) in 20 mM Tris-HCl buffer (pH7.2) containing 100 mM $MgCl_2$ and 1 μg/ml BSA. Reaction was monitored by excitation at 490 nm and emission at 522 nm.

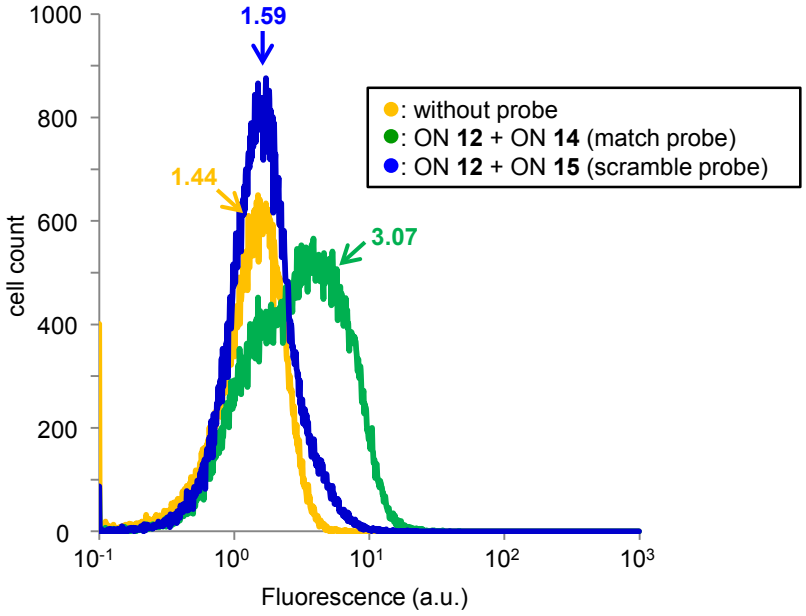


Figure S4. Detection of 23S rRNA in bacterial cells by flow cytometry. Flow cytometry histogram showing cell-count frequency vs fluorescence intensity for each probe. These histograms correspond to cells that were treated by no probe (negative control, orange); by scramble probe (blue) and by 23S rRNA targeted probes (green). The values on each histogram indicate the mean fluorescent signals.

Experimental details

Materials and General Instrumentation. General chemicals were purchased from Wako Pure Chemical, Tokyo Chemical Institute, Kanto Chemical or Sigma-Aldrich and were used without further purification. NMR spectra were recorded on a JEOL instrument at 400 MHz and 500 MHz for ^{1}H NMR and 100 MHz and 125 MHz for ^{13}C NMR.

Synthesis of N-(tert-Butoxycarbonyl)-N-(4-iodophenyl) piperidine-4-carboxamide (2). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.3130 g, 12.1 mmol, 1.2 eq) was added to a solution of compound **1**[1] (2.2790 g, 9.9 mmol) and 4-iodoaniline (2.4156 g, 11.0 mmol, 1.1 eq) in DMF (50 ml). The reaction mixture was stirred overnight. The mixture was diluted with EtOAc and washed with 2 N HCl and brine, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography to give the compound **2** (4.1708 g, 9.7 mmol, 98%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.62-7.60, 7.31-7.29 (each 2H, d, *J*= 8.8, 8.6 Hz), 7.35 (1H, s), 4.17 (2H, m), 2.77 (2H, m), 2.37 (1H, m), 1.89 (2H, m), 1.75 (2H, m), 1.46 (9H, s). ^{13}C-NMR (99.5 MHz, $CDCl_3$): δ 172.46, 154.49, 137.79, 137.42, 121.58, 87.47, 79.81, 77.21, 44.39, 28.62, 28.51. QSTAR (Applied Biosystems/MDS SCIEX) (ESI): $[M+Na]^+$ $C_{17}H_{23}IN_2NaO_3$: 453.0651, found: 453.0654.

Synthesis of N-(4-iodophenyl) piperidine-4-carboxamide hydrochloride (3). 4N HCl in dioxane (40 ml) was added dropwise to compound **2** (6.9057 g, 16.05 mmol) at 0 ˚C. The reaction mixture was stirred for 1h at room temperature. The solution was evaporated to afford compound **3** (5.0597 g, 13.80 mmol, 86%).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.36 (1H, s), 9.09-8.82 (2H, br), 7.64-7.62(2H, d, *J*= 8.8 Hz), 7.49-7.47(2H, d, J= 8.8Hz), 3.32-3.29 (2H, d, *J*= 12.4 Hz), 2.92-2.86 (2H, t,

J= 11.2 Hz), 2.70-2.65 (1H, t, *J*= 11.2 Hz), 1.97-1.94 (2H, m), 1.87-1.78 (2H, m). ^{13}C-NMR (99.5 MHz, DMSO-d_6): δ 172.30, 138.99, 137.27, 121.39, 86.63, 42.27, 39.93, 24.97. QSTAR (Applied Biosystems/MDS SCIEX) (ESI): $[M+H]^+$ $C_{12}H_{16}IN_2O$: 331.0302, found: 331.0296.

Synthesis of (E)-tert-butyl 4-(4-((4-(dimethylamino)phenyl)diazenyl)benzamido) cyclohexylcarbamate (5). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.253 g, 1.32 mmol, 2 eq), and triethylamine (2 drops) were added to a solution of compound **4**[2] (0.166 g, 0.78 mmol, 1.2 eq) and p-methyl red (0.177 g, 0.66 mmol) in CH_2Cl_2 (30 ml). The reaction mixture was stirred overnight. The mixture was diluted with CH_2Cl_2 and washed with 2 N HCl, water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography to give the compound **5** (80.9 mg, 0.17 mmol, 26%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.91-7.83 (6H, m), 6.77 (2H, d, *J*= 9.0 Hz), 6.12-5.98 (1H, m), 4.60-4.44 (1H, m), 4.12-3.98, 3.69-3.48 (each 1H, m), 3.11 (6H, s), 2.13-1.25 (8H, m), 1.46 (9H, s). ^{13}C-NMR (99.5 MHz, $CDCl_3$): δ166.13, 154.87, 152.65, 143.49, 134.66, 127.60, 125.29, 122.16, 111.37, 77.20, 48.29, 46.56, 40.35, 32.16, 31.94, 28.94, 28.52. QSTAR (Applied Biosystems/MDS SCIEX) (ESI): $[M+H]^+$ $C_{26}H_{36}N_5O_4$: 466.2818, found: 466.2805.

Synthesis of (E)-N-(4-aminocyclohexyl)-4-((4-(dimethylamino)phenyl)diazenyl) benzamide trifluoroacetate (6). Trifluoroacetic acid (6 ml) was added dropwise to compound **5** (80 mg, 0.17 mmol) at 0 ˚C, and the reaction solution was then returned to room temperature. The reaction mixture was stirred for 2 hours. After the addition of toluene, the solution was evaporated to afford compound **6** (132.6 mg).

^{1}H-NMR (400 MHz, CD_3OD): δ 7.93-7.83 (6H, m), 6.84-6.82 (2H, d, *J*= 9.0 Hz), 3.90, 3.11 (each 1H, m), 2.11, 1.54 (each 4H, m). ^{13}C-NMR (99.5 MHz, CD_3OD): δ167.08, 153.07, 142.69, 133.98, 127.85, 125.96, 121.34, 111.91, 77.44, 77.19, 47.53, 40.31, 29.98, 29.28. QSTAR (Applied Biosystems/MDS SCIEX) (ESI): $[M+H]^+$ $C_{21}H_{28}N_5O_2$: 366.2294, found: 366.2289.

Synthesis of 1-[2-hydroxy-2-(4-hydroxyphenyl)acetyl]-N-(4-iodophenyl)piperidine -4-carboxamide (7). 1-Ethyl-3-(3-dimethyl-aminopropyl)carbodiimide hydrochloride (0.6979 g, 3.64 mmol, 1.2 eq), 1-hydroxybenzotriazole (0.4915 g, 3.64 mmol, 1.2 eq) and triethylamine (418 µl, 1.0 eq) were added to a solution of DL-4-hydroxymandelic acid monohydrate (0.6333 g, 3.40 mmol, 1.1 eq) and compound **3** (1.1024 g, 3.00 mmol) in DMF (30 ml). The reaction mixture was stirred overnight. The mixture was diluted with CH_2Cl_2 and washed with 2 N HCl, water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography to give the compound **7** (1.2487 g, 2.60 mmol, 86%).

^{1}H-NMR (400 MHz, CD_3OD): δ7.59-7.57(2H, t, *J*= 8.5 Hz), 7.35-7.28(2H, dd, *J*= 28.8, 8.6 Hz), 7.22-7.18(2H, t, *J*= 7.8 Hz), 6.80-6.76 (2H, t, J= 8.2 Hz), 5.36-5.30 (1H, d, *J*= 23.7 Hz) 4.62-4.50(1H, dd, *J*= 47.1, 13.2 Hz), 3.94-3.87(1H, t, *J*= 12.9 Hz), 3.02-2.95(1H, t, *J*= 14.3 Hz), 2.77-2.71(1H, m), 2.52-2.50(1H, m), 1.84-1.81(1H, m), 1.71-1.59(1H, m), 1.52-1.49(1H, d, *J*= 12.4 Hz), 0.97-0.88 (1H, q, *J*= 11.5 Hz). ^{13}C-NMR (99.5 MHz, CD_3OD): δ174.98, 172.74, 158.76, 139.55, 138.67, 131.41, 129.67, 122.90, 116.59, 87.62, 72.63, 45.56, 44.56, 43.14, 29.42, 20.91. QSTAR (Applied Biosystems/MDS SCIEX) (ESI): $[M+Na]^+$ $C_{20}H_{21}IN_2NaO_4$: 503.0444, found: 503.0436.

Synthesis of 1-[2-hydroxy-2-(4-methylthiomethoxyphenyl)acetyl]-N-(4-iodophenyl) piperidine-4-carboxamide (8).[3] Sodium iodide (15.9 mg, 0.11 mmol, 0.18 eq) was added to a solution of compound **7** (0.2795 g, 0.58 mmol) in DMF (1.3 ml). After the reaction solution had been cooled at 0℃, potassium t-butoxide (97.2 mg, 0.86 mmol, 1.5 eq) dissolved in THF (1.5 ml) was added dropwise to the reaction mixture. Thereafter, chloromethylmethylsulfide (73 µl, 0.87 mmol, 1.5 eq) was added to the solution, and the obtained mixture was then stirred at room temperature. The reaction mixture was stirred for 2 h. The mixture was diluted with EtOAc and washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography to give the compound **8** (0.2467 g, 0.46 mmol, 78%).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.99-9.96 (1H, d, J= 13.9 Hz), 7.60, 7.43-7.35, 7.29-7.23, 6.98 (each 2H, m), 5.43-5.27 (1H, m), 5.43-5.27 (1H, m), 5.25 (2H, s), 2.15 (3H, s), 4.43-4.40, 3.97-3.92, 2.92, 2.76-2.65, 1.79, 1.63, 1.52-1.46, 1.34, 0.93 (each 1H, m). ^{13}C-NMR (99.5 MHz, DMSO-d_6): δ 172.69, 170.02, 155.92, 138.81, 137.05, 133.25, 127.70, 121.11, 115.64, 86.31, 71.69, 42.45, 41.29, 27.98, 20.78, 13.91. QSTAR (Applied Biosystems/MDS SCIEX) (ESI): $[M+Na]^+$ $C_{22}H_{25}IN_2NaO_4S$: 563.04777, found: 563.0469.

Synthesis of 1-[2-hydroxy-2-(4-azidomethoxyphenyl)acetyl]-N-(4-iodophenyl) piperidine-4-carboxamide (9). N-Chlorosuccinimide (0.8207 g, 6.01 mmol, 2.0 eq) was added to a solution of compound **8** (1.6362 g, 3.03 mmol) in CH_2Cl_2 (60 ml) at 0 ˚C. The reaction mixture was stirred for 5 minutes at 0 ˚C, then trimethylsilyl chloride (780 µl, 6.10 mmol, 2.0 eq). The reaction mixture was stirred for 1.5 h. The mixture was diluted with $CHCl_3$ and washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4 and evaporated. The residue was dissolved in DMF (30 ml) and a solution of

sodium azide (0.2386 g, 3.67 mmol, 1.2 eq) in water (3.6 ml) at 0 ˚C. The reaction mixture was stirred for 1.5 h at room temperature. The mixture was diluted with EtOAc and washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography to give the compound **9** (1.0805 g, 2.02 mmol, 67%). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.99-9.96(1H, d, *J*= 12.7 Hz), 7.60(2H, s), 7.43-7.37(2H, q, *J*= 7.6 Hz), 7.33-7.31(2H, q, *J*= 8.6 Hz), 7.03-7.02(2H, d, *J*= 6.4 Hz), 5.51-5.47(1H, m), 5.38(2H, s), 4.43-4.39(1H, d, *J*= 12.7 Hz), 4.06-3.93(1H, m), 2.95-2.89(1H, m), 2.80-2.62 (2H, m), 1.81-1.77(1H, m), 1.66-1.30(3H, m), 0.99-0.92 (1H, m). ^{13}C-NMR (99.5 MHz, DMSO-d_6): δ 172.64, 170.12, 155.24, 138.81, 137.04, 134.30, 127.94, 121.13, 115.54, 86.30, 78.78, 70.58, 59.69, 43.82, 42.44, 41.29, 27.92. QSTAR (Applied Biosystems/MDS SCIEX) (ESI): $[M+K]^+$ $C_{21}H_{22}IKN_5O_4$: 574.0354, found: 574.0335.

Synthesis of (E)-1-(4-(azidomethoxy)phenyl)-2-(4-(4-iodophenylcarbamoyl) piperidin-1-yl)-2-oxoethyl-(4-((4-(dimethylamino)phenyl)diazenyl)benzamido)cyclo hexylcarbamate (11). 4-Nitrophenyl chloroformate (129 mg, 0.64 mmol, 4 eq) and triethylamine (220 µl, 1.58 mmol, 9.9 eq) were added to the solution of compound **9** (84.9 mg, 0.16 mmol) in CH_2Cl_2 (8 ml). The reaction mixture was stirred for 3 h. The mixture was diluted with $CHCl_3$ and washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography to give the compound **10**.

Compound **10** was dissolved in CH_2Cl_2 (8 ml), and compound **6** (44.2 mg, 0.092 mmol, 0.5 eq) and triethylamine (100 µl, 0.717 mmol, 4.5 eq) were then added to the solution, followed by stirring overnight. Thereafter, the reaction solution was heated at 35°C for 2.5 h. The mixture was diluted with $CHCl_3$, and washed with water and brine,

dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography to give the compound **11** (52.8 mg, 0.057 mmol, 36% (2 steps)).

^{1}H-NMR (400 MHz, $CDCl_3$): δ7.89-7.85 (6H, t, *J*= 8.2 Hz), 7.59-7.57, 7.40-7.29, 7.04-7.02, 6.79-6.77 (each 2H, m), 6.25-6.22 (1H, d), 6.11 (1H, m), 5.19 (2H, s), 4.55 (1H, m), 3.94 (2H, m), 3.45-3.37 (4H, m), 3.12 (6H, s), 2.85-2.78 (2H, m), 2.47 (1H, m), 2.09 (4H, m), 1.91-1.62 (4H, m), 1.40 (6H, m). ^{13}C-NMR (99.5 MHz, $CDCl_3$): δ 172.76, 167.50, 166.93, 154.62, 152.62, 143.18, 137.84, 137.41, 134.38, 129.42, 127.67, 125.11, 121.76, 121.40, 116.13, 111.25, 86.97, 79.32, 72.17, 44.40, 42.83, 42.09, 40.09, 31.21, 29.58, 28.01. QSTAR (Applied Biosystems/MDS SCIEX) (ESI): $[M+H]^+$ $C_{43}H_{48}IN_{10}O_6$: 927.2803, found: 927.2801.

Synthesis of (E)-1-(4-(azidomethoxy)phenyl)-2-(4-(4-(3-(2-bromoacetamido) prop-1-ynyl)phenylcarbamoyl)piperidin-1-yl)-2-oxoethyl-4-(4-((4-(dimethylamino) phenyl)diazenyl) benzamido)cyclohexylcarbamate (12). Tetrakis(triphenylphos-phine)palladium (6.6 mg, 0.006 mmol, 0.1 eq), copper(I) iodide (2.0 mg, 0.011 mmol, 0.2 eq) and triethylamine (40 µl, 0.287 mmol, 5.0 eq) were added to a mixture of compound **11** (52.8 mg, 0.057 mmol) and 2-bromo-N-(propargyl) acetamide[4] (52.8 mg, 0.302 mmol, 5.3 eq) in DMF (1.1 ml). The reaction mixture was stirred for 0.5 h. The solution was evaporated and the residue was purified by flash chromatography to give the compound **12** (48.1 mg, 0.049 mmol, 87%).

^{1}H-NMR (400 MHz, DMSO-d_6): δ10.09-10.02 (1H, d, *J*= 26.1 Hz), 8.80, 8.33 (each 1H, m), 7.83-7.79 (6H, t, *J*= 7.9 Hz), 7.63-7.61, 7.47-7.45, 7.33,7.07 (each 2H, m), 6.86-6.84 (2H, d, *J*= 9.0 Hz), 6.24-6.20 (1H, d, *J*= 15.4 Hz), 5.41 (2H, s), 4.37 (1H, m), 4.14-4.03 (4H, m), 3.89 (2H, s), 3.73-3.67 (1H, m), 3.32 (4H, br), 3.11 (6H, s), 2.89-2.68 (1H, m), 1.86-1.57 (6H, m), 1.42-1.23 (6H, m). ^{13}C-NMR (99.5 MHz,

DMSO-d_6): δ 165.49, 164.58, 153.60, 152.56, 142.39, 134.74, 131.79, 129.64, 128.18, 124.85, 121.20, 118.76, 115.66, 111.40, 78.68, 31.36, 30.91, 29.26, 29.09, 25.51, 0.15. QSTAR (Applied Biosystems/MDS SCIEX) (ESI): $[M+H]^+$ $C_{48}H_{53}BrN_{11}O_7$: 974.3313, found: 974.3301.

Synthesis of 1-(4-(azidomethoxy)phenyl)-2-(4-(4-iodophenylcarbamoyl) piperidin-1-yl)-2-oxoethyl((3R,4R,5S,6S)-3,4,5-trihydroxy-6-(isopropylthio)tetrahydro-2H-pyran-2-yl)methylcarbonate (13). 4-Nitrophenyl chlorofarmate (96.3 mg, 0.48 mmol, 5.0 eq) and triethylamine (130 µl, 0.93 mmol, 9.8 eq) were added to a solution of compound **9** (51.2 mg, 0.10 mmol) in CH_2Cl_2 (5 ml). The reaction mixture was stirred for 4h. The mixture was diluted with $CHCl_3$ and washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography to give the compound **10**.

Isopropyl β-D-1-thiogalactopyranoside (21.6 mg, 0.09 mmol, 0.9 eq) and DMAP (11.0 mg, 0.09 mmol, 0.9 eq) were added to a solution of compound **10** in pyridine (1 ml). The reaction mixture was stirred overnight. The mixture was diluted with EtOAc and washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography to give the compound **13** (22.3 mg, 0.03 mmol, 29% (2steps)).

^{1}H-NMR (500 MHz, DMSO-d_6): δ10.01-9.94(1H, d, *J*= 36.2 Hz), 7.61-7.56(2H, q, *J*= 8.9 Hz), 7.48-7.42(3H, m), 7.36-7.35(1H, d, *J*= 8.7 Hz), 7.10-7.06(2H, t, 9.4 Hz), 6.31-6.25(1H, m), 5.40(1H, s), 4.93-4.91(1H, t, *J*= 5.5 Hz), 4.88-4.86(1H, d, *J*= 8.7 Hz), 4.71-4.69(1H, m), 4.64-4.49(1H, m), 4.38-4.30(2H, m), 4.15-4.07(1H, m), 4.01-3.87(1H, m), 3.73-3.59(2H, m), 3.50-3.43(1H, m), 3.12-3.04(2H, m), 2.79-2.59(1H, m), 1.80-1.44(4H, m), 1.24-1.14 (7H, m). ^{13}C-NMR (125.8 MHz,

DMSO-d_6): δ 172.90, 156.72, 153.86, 139.06, 137.26, 130.04, 127.65, 121.36, 115.83, 86.43, 84.96, 79.18, 78.74, 75.70, 74.21, 69.97, 68.68, 60.31, 44.03, 42.50, 41.34, 33.78, 27.81, 23.87. QSTAR (Applied Biosystems/MDS SCIEX)(ESI-Q-TOF): $[M+Na]^+$ $C_{31}H_{36}BrN_5NaO_{10}S$: 822.1276, found: 822.1299.

Synthesis of 1-(4-(azidomethoxy)phenyl)-2-(4-(4-(3-(2-bromoacetamido)prop-1-ynyl)phenylcarbamoyl)piperidin-1-yl)-2-oxoethyl((3R,4R,5S,6S)-3,4,5-trihydroxy-6-(isopropylthio)tetrahydro-2H-pyran-2-yl)methylcarbonate (14). Tetrakis(triphenylphosphine)palladium (5.4 mg, 0.005 mmol, 0.1 eq), copper(I) iodide (2.1 mg, 0.01 mmol, 0.3 eq) and triethylamine (24 µl, 0.17 mmol, 5.0 eq) were added to a mixture of compound **13** (27.7 mg, 0.053 mmol) and 2-bromo-N-(propargyl) acetamide (31.6 mg, 0.18 mmol, 5.2 eq) in DMF (0.7 ml). The reaction mixture was stirred for 1h. The solution was evaporated and the residue was purified by flash chromatography to give the compound **14** (19.3 mg).

QSTAR (Applied Biosystems/MDS SCIEX)(ESI-Q-TOF): $[M+Na]^+$ $C_{36}H_{43}BrN_6NaO_{11}S$: 869.1786, found: 869.1767.

Synthesis of unmodified oligonucleotides. Oligonucleotides were synthesized on a 0.2 µmol scale on a DNA/RNA synthesizer (H-8-SE; Gene World) using standard phosphoroamidite coupling chemistry. Deprotection and cleavage from the CPG support was carried out by incubation in concentrated ammonia for 4 hours at 55 °C. Following deprotection, the oligonucleotides were purified by reverse-phase column chromatography (MicroPure II column; Biosearch Technologies) and quantitated by UV absorbance using the nearest-neighbor approximation to calculate molar absorptivities.

Synthesis of 3'-methyl red linked oligonucleotide (ON 1 or 12). The bromoacetyl group of compound **12** was reacted with the phosphorothioate group on the DNA. For

3'-phosphorothioate sequences, the 3'-phosphate CPG was sulfurized by the sulfurizing reagent (Glen Research) after the first nucleotide was added. The reaction was carried out by intensively stirring a mixed solution comprising 3 mM compound **12** (in DMF), 30 mM NaB buffer (pH 8.5) and 300 μM 3'-phosphorothioate oligonucleotides solution at room temperature for 5 h (DMF concentration in the reaction solution: 60%). The reacted products were collected by ethanol precipitation. Next, the products were purified by reverse phase HPLC (gradient conditions: 0%-60% acetonitrile/50 mM triethylammonium acetate). The probe structure was confirmed by MALDI-TOF mass spectrometry. ON **1**: calculated mass, $C_{175}H_{201}N_{49}O_{76}P_{10}S$ 4546.05; found 4561.70.; ON **12**: calculated mass, $C_{223}H_{259}N_{70}O_{108}P_{15}S$ 6141.27; found 6172.21.

Synthesis of 3'-IPTG linked oligonucleotide (ON 13). The reaction was carried out by intensively stirring a mixed solution comprising 3 mM compound **14** (in DMF), 80 mM TEAA buffer (pH 7.0) and 200 μM 3'-phosphorothioate oligonucleotides solution at room temperature for 5 h (DMF concentration in the reaction solution: 80%). The reacted products were collected by ethanol precipitation. Next, the products were purified by reverse phase HPLC (gradient conditions: 0%-60% acetonitrile/50 mM triethylammonium acetate). The probe structure was confirmed by MALDI-TOF mass spectrometry. ON **13**: calculated mass, $C_{183}H_{227}N_{63}O_{105}P_{15}S_2$ 5515.0; found 5519.4.

Synthesis of 5'-triphenylphosphine (TPP) linked oligonucleotide (OD 2-4, 14, 15). Carboxy-triphenylphosphine (TPP) NHS ester was reacted with 5'-amino-modified oligonucleotide. 5'-aminomodifier C6 (Glen Research) was used to prepare 5'-aminomodified oligonucleotide. The reaction was carried out by intensively stirring a mixed solution comprising 8 mM TPP NHS ester, 50 mM sodium tetraborate (pH 8.5) and 200 μM 5'-amino-modified oligonucleotides solution at room temperature for 5

hours (DMF concentration in the reaction solution: 46%). The reacted products were collected by ethanol precipitation. Next, the collected products were purified by reverse-phase HPLC (0-60% acetonitrile/50 mM triethylammonium acetate gradient). The probe structure was confirmed by MALDI-TOF mass spectrometry.

ON **2**: calculated mass, $C_{124}H_{150}N_{43}O_{63}P_{11}$ 3589.70; found 3590.64.

ON **3**: calculated mass, $C_{104}H_{126}N_{33}O_{52}P_{9}$ 2947.59; found 2948.68.

ON **4**: calculated mass, $C_{114}H_{138}N_{38}O_{57}P_{10}$ 3260.64; found 3261.59.

ON **13**: calculated mass, $C_{169}H_{214}N_{46}O_{98}P_{16}$ 4950.90; found 4955.39.

ON **14**: calculated mass, $C_{169}H_{212}N_{52}O_{95}P_{16}$ 4984.92; found 4989.44.

A peak corresponding to the oxidized product (+O) was also seen and presumed to arise from oxidation during purification (ON **13** and **14**).

Reaction of ON 1 with DTT. Reactions with DTT were performed in 1.2 ml of Tris-HCl buffer (20 mM, pH 7.2) with ON **1** (50 nM) and DTT (100 mM) at 37°C. Reactions were observed by fluorescence spectrometry (FP-6500, JASCO). Fluorescence was measured after 0, 30, 60, 120 and 180 minutes have passed. An excitation wavelength was set at 490 nm.

DNA-templated reaction. Reactions on the DNA template were performed in 1.2 ml of Tris-HCl buffer (20 mM, pH 7.2) containing 100 mM $MgCl_2$ and 1.0 μg/ml BSA with target DNA (ON **5-9**, 50 nM), ON **1** (50 nM), and ON **4** (250 nM) at 37°C. The increase of fluorescence intensity produced by reduction of ON **1** was continuously monitored at time intervals. Reactions were observed by fluorescence spectrometry. For the time course of the azide reduction, the fluorescence intensity was measured for 0.5 s at 1 min intervals: excitation, 490 nm; emission, 522 nm.

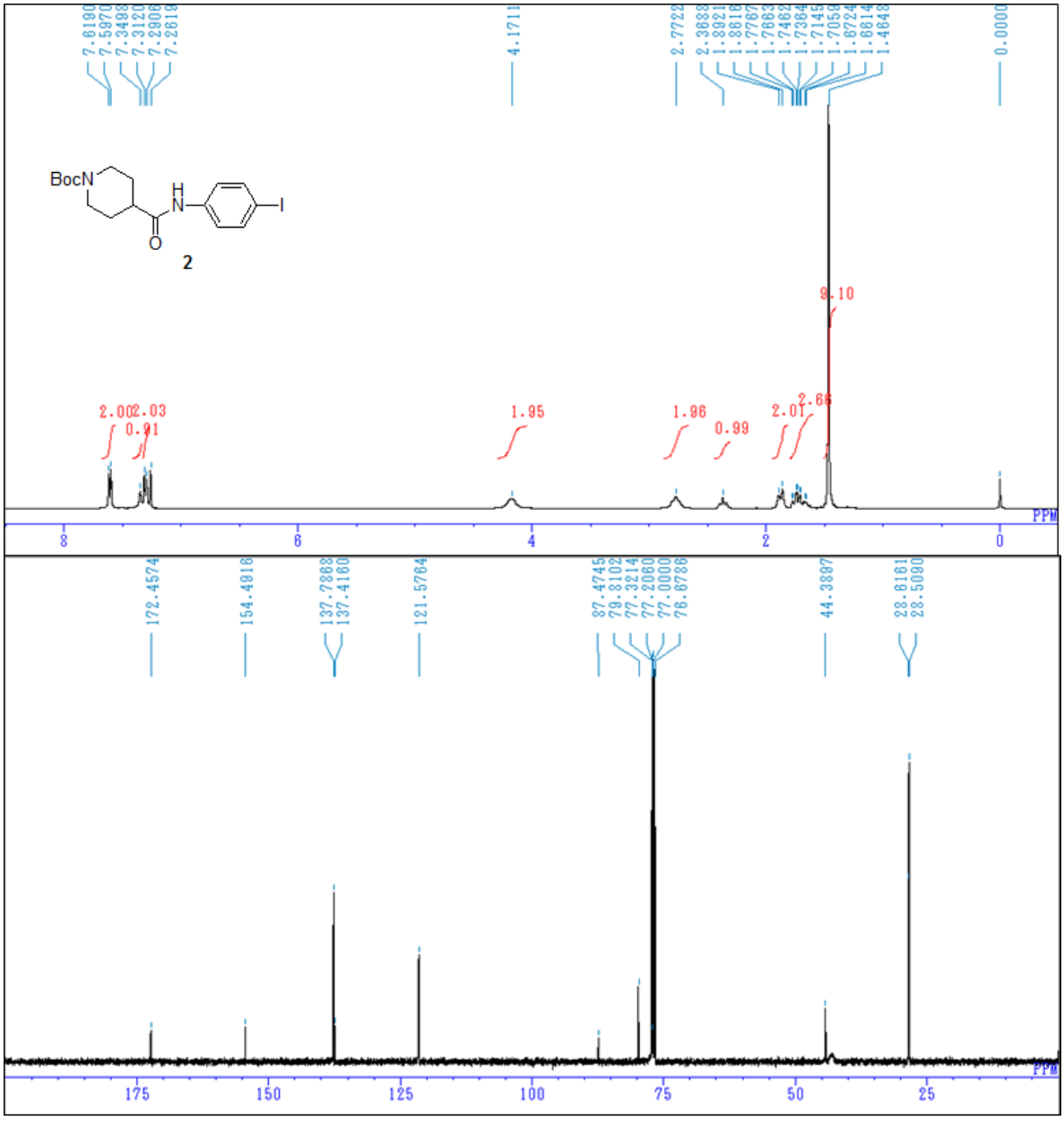
Introduction of probe into *Escherichia coli.* *E. coli* JM109(DE3) was pre-cultured at 37°C in an LB/Amp medium overnight. A cell mass was recovered so that OD_{600}= 1.0 (1 ml volume), and the cells were centrifuged and were then suspended in 250 µl of a buffer (0 or 200 nM probes (ON **12**, and ON **14** or **15**), 20 mM Tris-HCl (pH 7.2), 0.9 M NaCl, and 0.1% SDS). The suspension was incubated at 37°C for 60 min, and the cells were centrifuged and were then suspended in 500 ml PBS buffer.

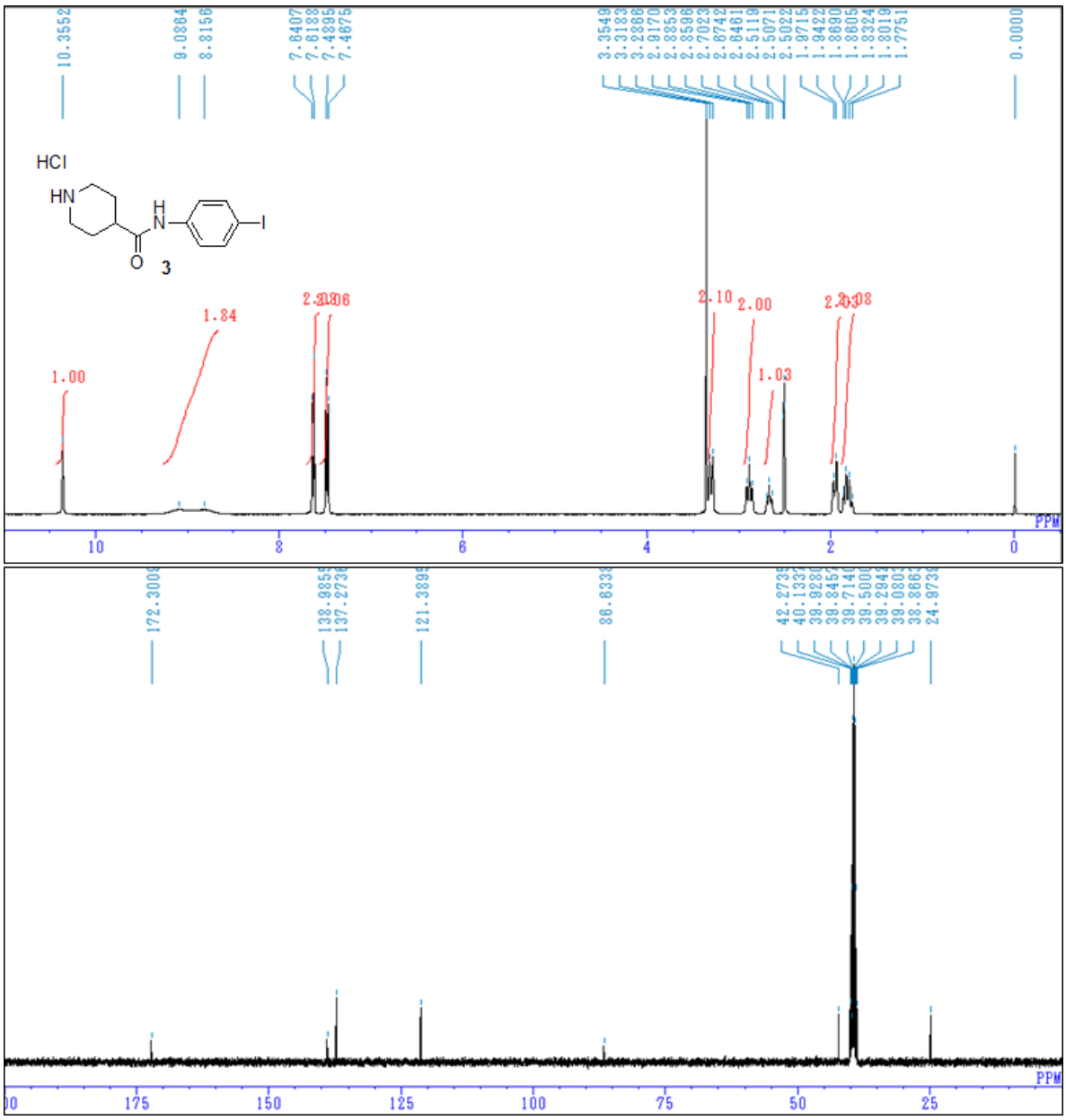
Expression of GFP using probes in *Escherichia coli.* pET-GFP vector (The about 700 bp NcoI-EcoRI fragment of the pAcGFP1 plasmid (Clontech) was inserted into pET-21d(+)) transformed *E. coli* JM109(DE3) was pre-cultured at 37°C in an LB/Amp medium overnight. A cell mass was recovered so that OD_{600}= 0.6 (50 µl volume), and the cells were centrifuged and were then suspended in 50 µl of a buffer (0 or 10 µM probes (ON **13**, and ON **14** or **15**), 20 mM Tris-HCl (pH 7.2), 0.9 M NaCl, and 0.1% SDS). The suspension was incubated at 37°C for 30 min, and the cells were centrifuged and were then suspended in 50 µl of an LB/Amp medium. Positive control suspended in LB/Amp medium containing 10 µM IPTG. The suspended culture was incubated to shaking and stirring at 37°C. 24 h later, the cell was recovered, followed by an FACS measurement.

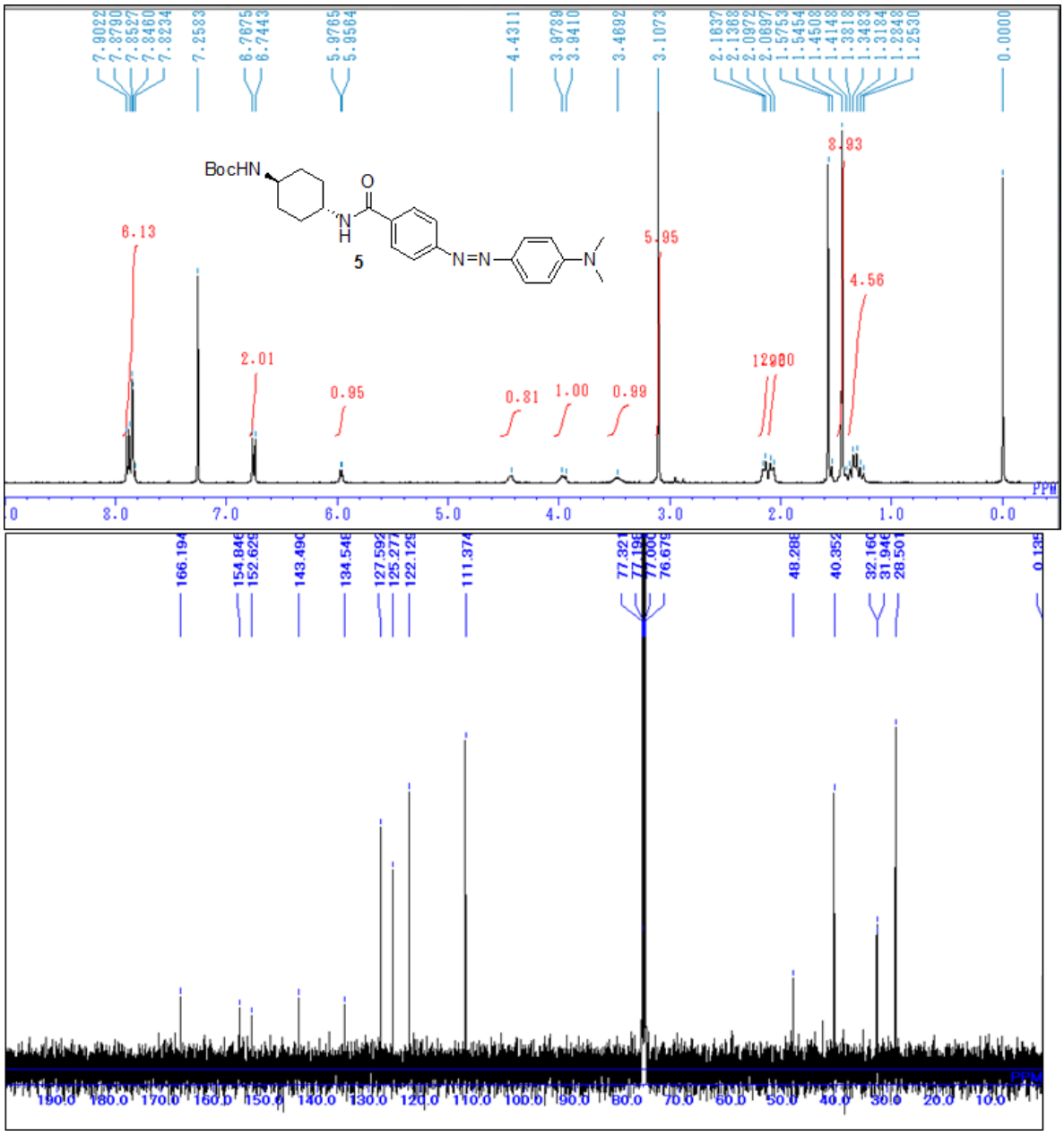
Flow cytometry. The live cell suspension was directly analyzed without any washing step with a Cytomics FC500 instrument (Beckman Coulter). Fluorescent signals were observed under the following conditions: excitation by argon laser, 488 nm; emission, 515-535 nm. Forward angle light scatter (FSC), side angle light scatter (SSC), and fluorescence data were recorded, and for each measurement, above 200,000 events were stored. Data were analyzed with the CXP analysis software (Beckman Coulter).

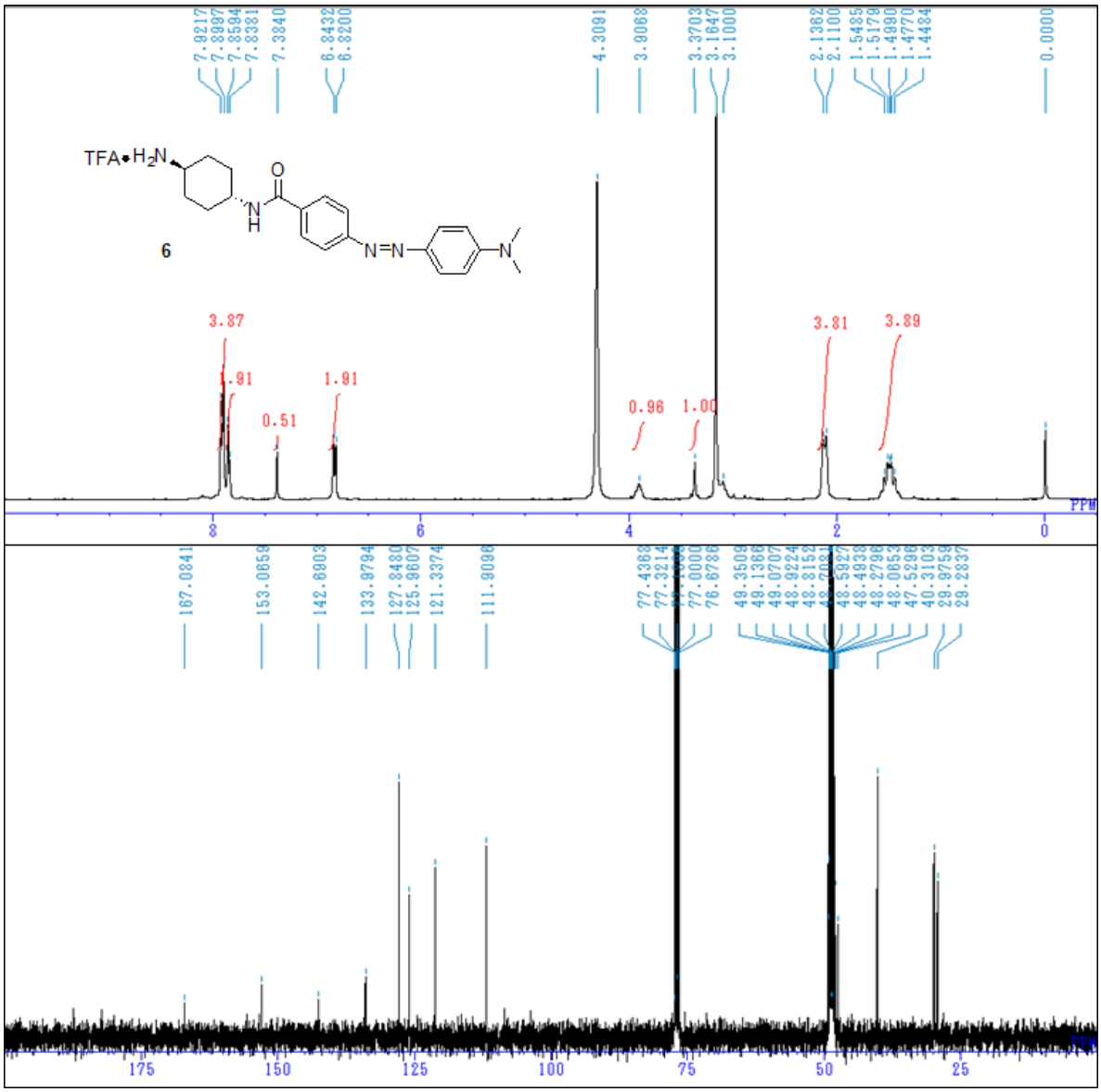
Fluorescent intensity was determined as the mean of fluorescent value of single cells lying in a gate that were defined in a FSC vs. SSC dot plot.

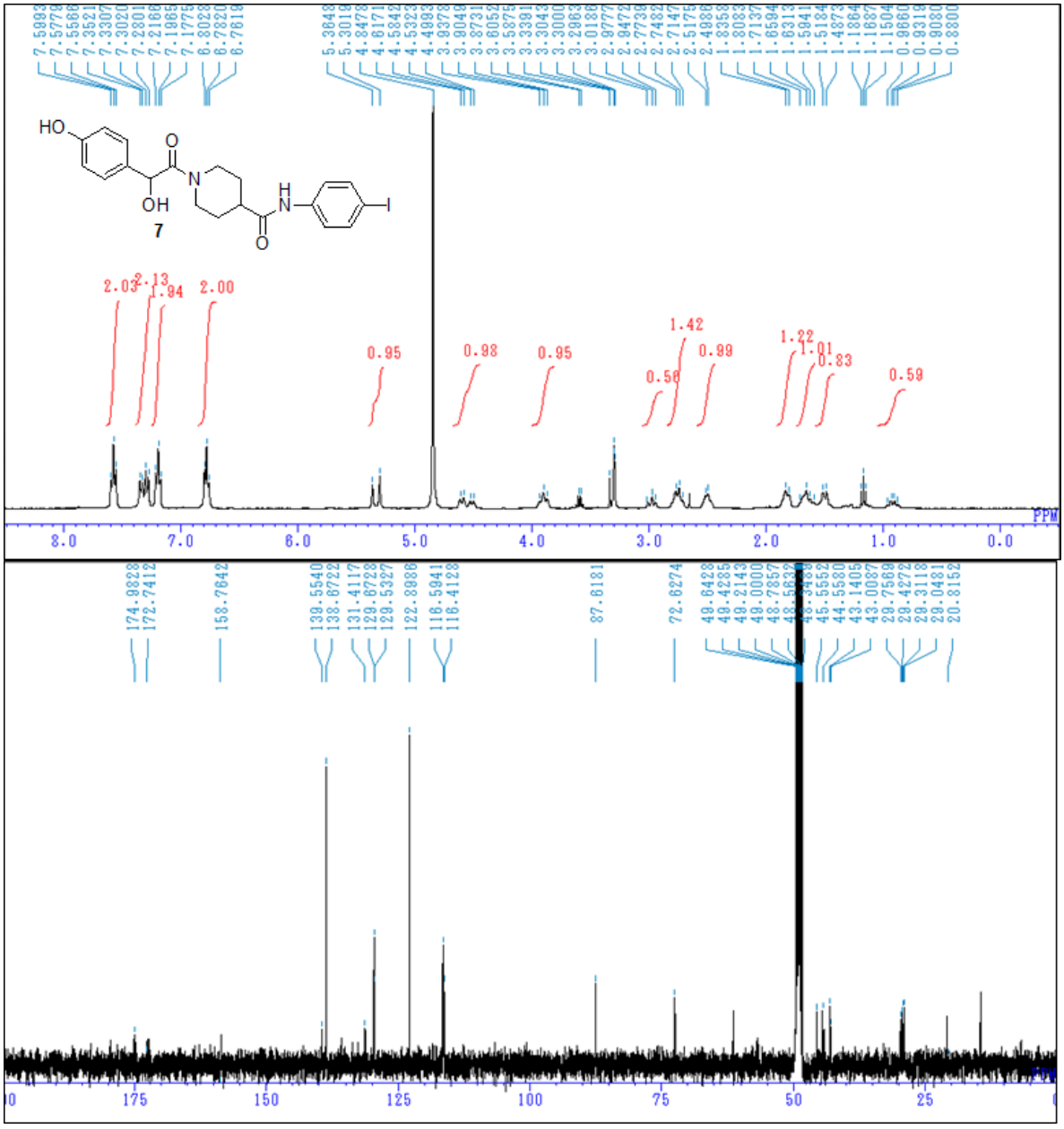
Imaging of GFP in *E. coli* cells. Deposit a drop of cell solution on a microscope slide and allow it to dry. Sample was put a drop of VECTASHIELD (H-1000, Vector Laboratories) and covered with the cover glass. Fluorescence images were obtained using a fluorescence microscope (FV1000-D, Olympus) and imaging software (FV1000 Viewer Installation). Microscope settings were as follows: excitation 488 bandpass filter; emission 500/10 bandpass filter.

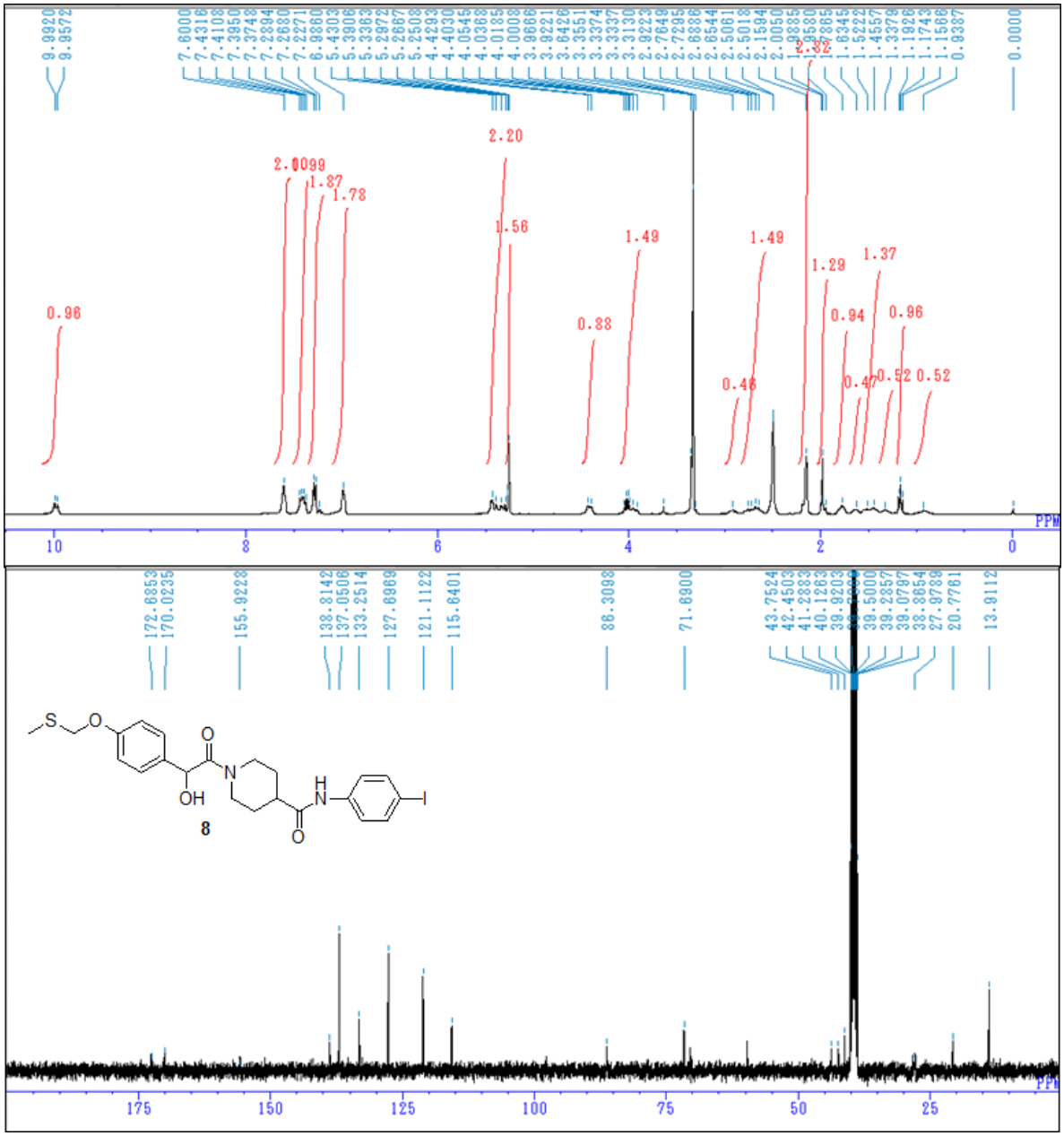


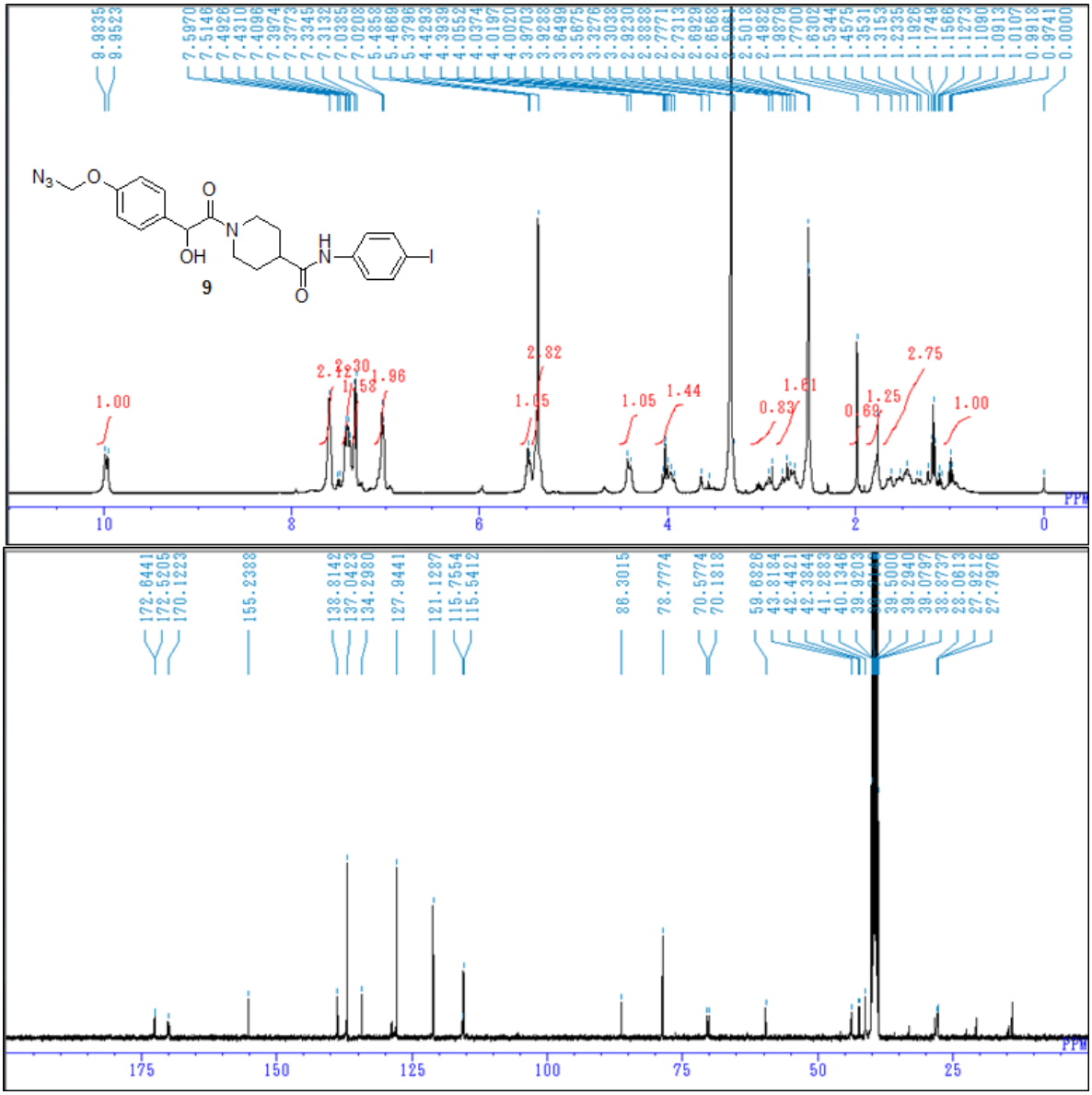


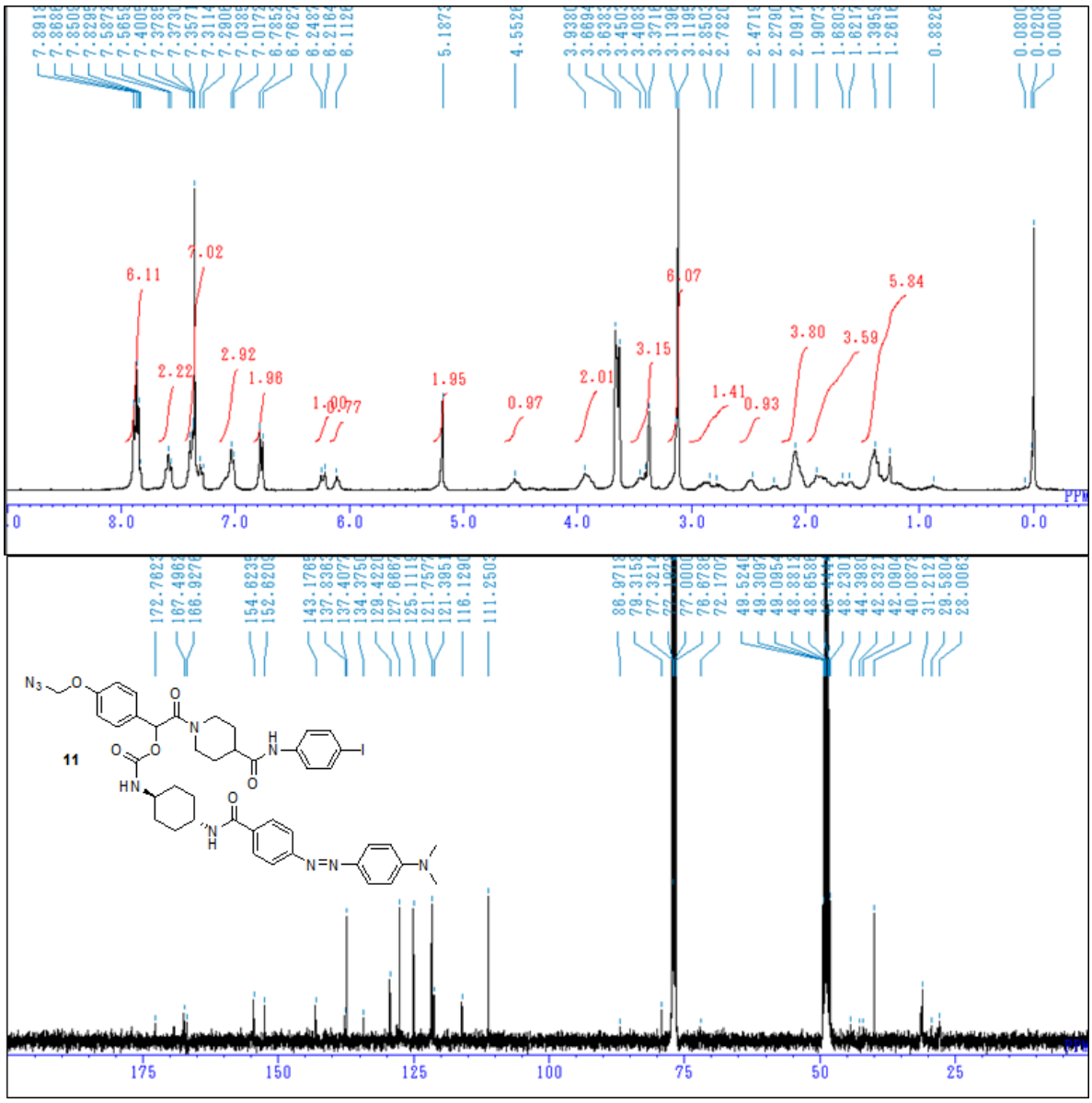


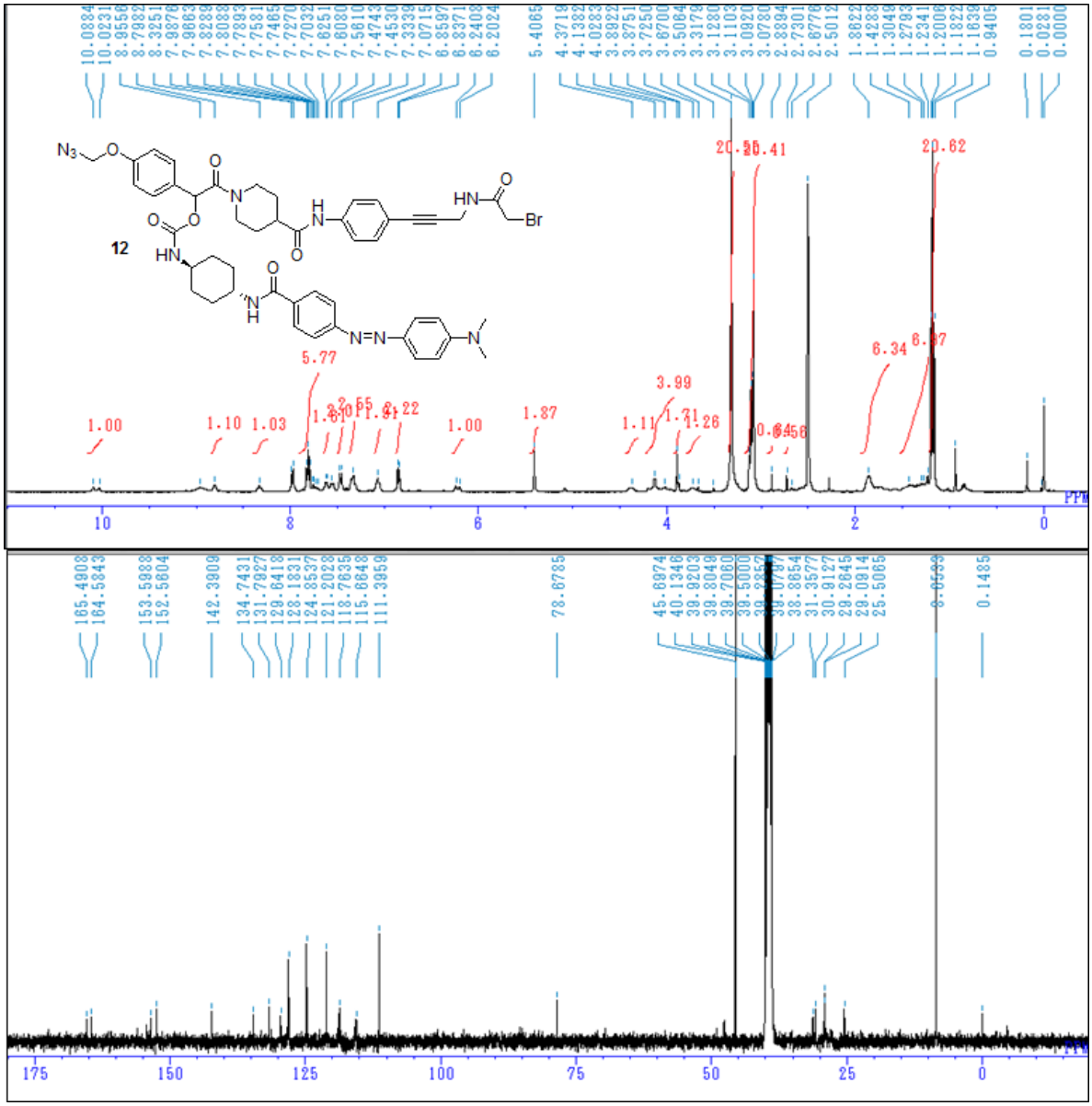


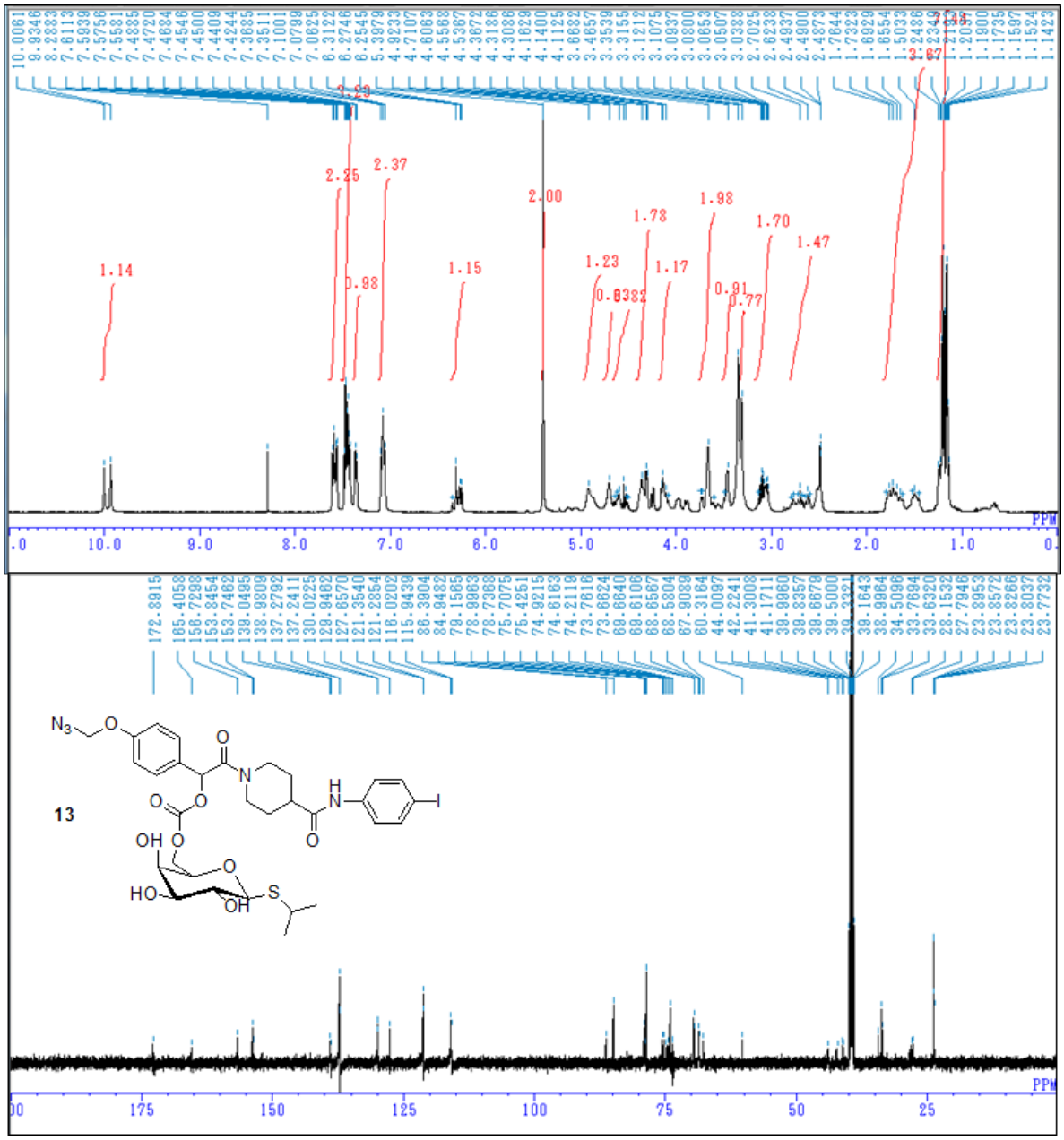












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